EXHIBIT 46

Pilot Evaluation of Venlafaxine Hydrochloride for the Therapy of Hot Flashes in Cancer Survivors

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Purpose: **Hot flashes can be a prominent clinical problem for breast cancer survivors and men who undergo androgen-deprivation therapy. Anecdotal information suggested a low dose of a relatively new antidepressant, venlafaxine, could abrogate this clinical problem.**

Materials and Methods: **This study included 28 consecutive assessable patients entered onto a phase II clinical trial. Hot flash data were collected by daily diary questionnaires during a 1-week baseline period and then for 4 weeks, during which time patients received venlafaxine 12.5 mg orally twice daily.**

Results: **Fifty-eight percent of patients who completed the study had a greater than 50% reduction in hot flash scores (frequency times severity) during the fourth treatment week as compared with the baseline week. Median weekly hot flash scores were reduced by 55% from baseline during the fourth week of venlafaxine therapy. Therapy was generally well tolerated and appeared to alleviate fatigue, sweating, and trouble sleeping.**

Conclusion: **Venlafaxine appears to represent an efficacious new method to alleviate hot flashes. Further evaluation of this compound for alleviating hot flashes is indicated.**

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IT IS WELL RECOGNIZED that hot flashes are a significant clinical problem in menopausal women. Estrogens, the mainstay of therapy for this symptom in most menopausal women, are generally contraindicated in women with a history of breast cancer.[1] A number of nonhormonal antidotes, such as clonidine, vitamin E, methyldopa, and belladonna alkaloids, have been proposed for treating menopausal or tamoxifen-induced hot flashes, but they are not very efficacious and/or have concomitant untoward side effects.[2-5]

Hot flashes can also be a significant clinical problem in men treated with androgen-deprivation therapy for prostate cancer.[6] Clonidine has been tested in this situation, but it does not appear to be efficacious.[7] Although estrogens have also been used for this problem, side effects can be problematic.

A recent placebo-controlled crossover clinical trial demonstrated that megestrol acetate, a progestational agent, decreases hot flashes by approximately 80% both in female breast cancer survivors and in men with hot flashes following androgen-deprivation therapy,[8] hence providing an alternative to estrogenic agents for treating this condition.

The safety of low-dose progestational agents in women with breast cancer is open to question. To date, there are no convincing data that low-dose progestational agents are harmful in such patients. Nevertheless, low-dose progesterone theoretically might stimulate the growth of metastatic breast cancer, potentiate the development of new primary breast cancers, or interfere with tamoxifen therapy. In addition, megestrol acetate, while successful, does not effectively alleviate hot flashes in all patients in whom it is used and may be associated with untoward side effects in some patients.[9] Hence, it is appropriate to identify other agents to alleviate hot flashes in cancer survivors.

Venlafaxine hydrochloride is a structurally novel antidepressant that inhibits neuronal serotonin and norepinephrine re-uptake. Recommended doses for treating depression range from 75 to 375 mg/d. Based on anecdotal experience that suggested low-dose (subtherapeutic) venlafaxine was efficacious for decreasing hot flashes, the present pilot trial was developed to investigate this possibility further.

MATERIALS AND METHODS

Patients considered for this clinical trial included women with a history of breast cancer who were currently without evidence of cancer and men who had androgen-deprivation therapy that was scheduled to last for at least 6 weeks beyond the date of trial entry. Furthermore, patients must have had bothersome hot flashes present for at least the prior month, the hot flashes must have been occurring at least 14 times per week, and they must have been of sufficient severity to make the patient desire therapeutic intervention. Life expectancy was to be greater than 6 months and the patient had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Patients must not have been receiving, or be scheduled to receive, any of the following: antineoplastic chemotherapy, androgens, estrogens,

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progestational agents, or antidepressants. Tamoxifen was allowed, but the patient must have been on a constant dose for at least 6 weeks and have been scheduled to continue tamoxifen during the 5-week study period. Patients must not have been receiving (or planned to receive) other agents for treating hot flashes during the study period.

After meeting all study requirements, patients were registered onto the study and were asked to complete a pen-and-paper daily diary for 1 week while not receiving any study medication. The daily diary was akin to those used in several previous studies.[2,5,7,8] Following the 1-week baseline period, patients were to receive venlafaxine at an oral dose of 25 mg/d administered as half of a scored 25-mg tablet twice daily. This low dose was chosen as anecdotal experience suggested that it had substantial activity against hot flashes. Patients were to complete daily diaries for the subsequent 4 weeks. Each patient was to be contacted by telephone by the study nurse during the second and fifth week to document compliance, to encourage completion of questionnaires, and to address problems.

Statistical Methods

Previous experience has demonstrated repeatedly that there is a relatively consistent 20% to 30% reduction of hot flashes achieved over 4 weeks' time with a placebo.[2,5,7,8] We wished to investigate whether venlafaxine would be associated with a hot flash reduction of a magnitude that was more than the expected placebo effect. The results of these observations were to serve as the basis to pursue, or abandon, a planned, placebo-controlled comparative trial using this agent.

The efficacy measures were the average number of daily hot flashes over each week, the hot flash score for each week (defined as the number of mild hot flashes for the week plus twice the number of moderate hot flashes plus three times the number of severe hot flashes plus four times the number of very severe hot flashes), the proportion of patients who reported substantial reduction (≥ 50%) in hot flash score, the proportion of patients who thought the medication was helping them, and the proportion of patients who wanted to continue taking the medication. A meta-analysis to compare results of the venlafaxine pilot study with previous hot flash data was accomplished by standardizing results from each trial into Z scores to control for intertrial variability. Toxicity data were recorded and changes in incidence from baseline to study completion were tested by McNemar's test.

RESULTS

The presented results were derived from 31 consecutive patients who entered this pilot study from July 1, 1997 to October 1, 1997. Two patients never started their study tablets and another went on a trip, was lost for follow-up evaluation during the study period, and did not return any study data. This left 28 assessable patients. The characteristics of the 28 assessable patients are listed in Table 1. Twenty-five patients completed the entire 5-week protocol period. Three patients did not complete this 5-week evaluation period. Two of these three patients stopped their study medications because of perceived toxicity (a decreased ability to concentrate or think clearly in one patient and the following symptoms in the second patient: depression, nausea, dry mouth, fatigue, and sleepiness). One additional patient quit because he decided to try megestrol acetate after 3 days of venlafaxine.

Table 1. Baseline Data

Variable	% of Patients (n = 28)
Age, years	
18-49	11
≥ 50	89
Sex	
Male	18
Female	82
Tamoxifen use	
Yes	68
No	32
Duration of hot flash symptoms (months)	
< 9	50
≥ 9	50
Average frequency of hot flashes (per day)	
2-3	11
4-9	61
≥ 10	29

At baseline, patients reported an average of 6.6 hot flashes per day (range, 3.1 to 33.9). After the first week of treatment, the average number of hot flashes reduced to 5.7 and by the end of the 5-week study period, patients reported an average of 4.3 hot flashes per day (range, zero to 19). Eight patients (29%) reported their hot flashes were reduced in number to fewer than two per day by study end. Fifteen of 28 patients (54%) reported a decrease in hot flash number of ≥ 50%. The incidence of severe and very severe hot flashes was reduced from an average of 1.4 per day at baseline to 0.1 per day by the end of the study ($P < .0002$).

To examine the venlafaxine effect on the combination of both hot flash frequency and severity, we used hot flash scores (frequency times average severity). The median changes in hot flash scores for patients during the 4 weeks of venlafaxine, compared with the data from the baseline week, are illustrated in Fig 1. A decrease in hot flash activity was seen in all but five patients in the first week of treatment.

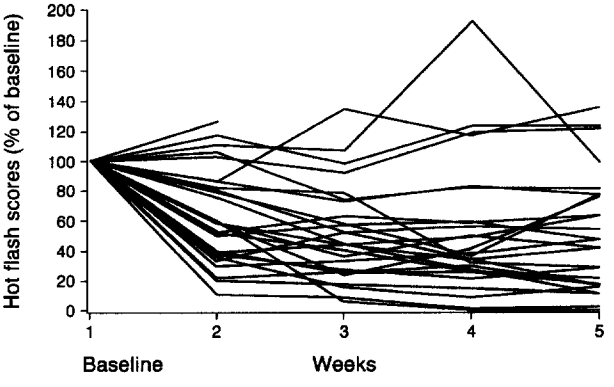

Fig 1. Stream plots of weekly hot flash scores for the individual patients on this clinical trial (n = 27).

After the first week, the hot flash activity seemed to stabilize to the end of the trial.

Understanding the limitations of cross-study comparisons, we compared efficacy data for venlafaxine with three previously conducted, placebo-controlled trials.[2,5,8] Figure 2 displays a remarkable consistency in the data from the three placebo arms in our previous trials, with median hot flash score reductions from baseline to the fourth week of approximately 20%. Hot flash scores were reduced by approximately 30% with the use of either vitamin E or clonidine. Hot flash scores were reduced by approximately 55% and 85% with venlafaxine and megestrol acetate, respectively. There appears to be a differential reduction in hot flashes during the first week between megestrol acetate, vitamin E, and venlafaxine. Venlafaxine appears to cause more abrupt declines in hot flashes than occurred with megestrol acetate or vitamin E. In this regard, 80% of the eventual decrease in hot flashes with venlafaxine was seen in the first week of therapy (44% decrease during the first week *v* 55% decrease by the fourth week) compared with megestrol acetate, where only 12% of the eventual decrease was seen the first week (8% of 85%). Further analysis of these data was performed by calculating single summary statistics for all patients on these trials who received placebo. The mean and standard deviation were used to produce Z scores for the efficacy results on each patient relative to the placebo effect. Results indicated that there was intertrial variability in terms of baseline hot flash activity. Once this variability had been accounted for, the basic findings of the nonstandardized analysis were supported. Hot flash changes from baseline data are also listed in Tables 2 and 3 for the current study and the three preceding studies. These again demonstrate a substantial hot flash reduction for patients who received venlafaxine (compared with placebo arms on the three previous trials).

The data from these trials were examined based on subject sex. While the small patient numbers prevent any firm conclusions based on such subsets, there did not appear to be

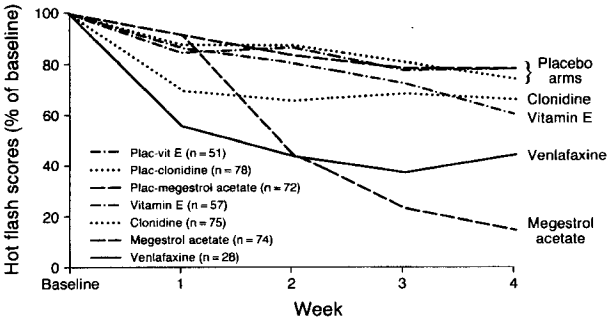

Fig 2. Weekly hot flash scores in patients who received venlafaxine *v* data from patients on 3 previous placebo-controlled trials.

Table 2. Percent of Baseline Hot Flash Scores During the Fourth Week of Therapy

	No.	0-24 (%)	25-49 (%)	50-74 (%)	75-100 (%)	> 100 (%)	N/A*
Placebo[2]	78	14	10	26	23	27	18
Placebo[5]	50	20	16	8	22	34	7
Placebo[8]	65	8	11	25	35	22	17
Clonidine[2]	73	15	23	23	21	18	23
Vitamin E[5]	52	15	19	23	17	25	11
Megestrol acetate[8]	67	66	18	10	6	0	14
Venlafaxine	25	35	23	12	19	12	3

*Data not available for reasons such as patients did not stay on study long enough or data were incomplete.

any qualitative differences between men and women involved in the trial.

To evaluate for toxicity, we asked patients to note whether they were having any of the following symptoms during each study week: appetite loss, sleepiness, nausea, constipation, dizziness, tiredness (fatigue), dry mouth, abnormal sweating, trouble sleeping, or nervousness. Keeping in mind that two patients stopped taking venlafaxine after 1 to 2 weeks because of perceived toxicity, we did not see any other suggestion of any changes in the first five of these symptoms when we compared the symptoms noted on the fourth week of venlafaxine versus symptoms that were present during the baseline week (when no medication was prescribed). However, interestingly, we did see rather striking decreases in the latter five symptoms during the treatment period (Table 4).

Overall, 19 of 28 (68%) patients thought that venlafaxine helped reduce hot flash activity and 18 of 28 (64%) wished to continue taking the medication beyond the end of the study.

DISCUSSION

The results from this prospective clinical trial support our prestudy hypothesis that low-dose venlafaxine reduces hot flashes to a greater degree than would be expected with a placebo.

We currently are aware that anecdotal observations, similar to those that preceded the present trial, have led to

Table 3. Median Hot Flash Score Reductions During the Fourth Week of Therapy (with 95% confidence intervals)

Variable	% Reduction	95% Confidence Interval
Placebo[2]	25	8-32
Placebo[5]	21	– 9-33
Placebo[8]	21	8-23
Clonidine[2]	33	21-44
Vitamin E[5]	39	0-46
Megestrol acetate[8]	85	72-87
Venlafaxine	55	22-71

Table 4. Toxicity Symptoms

Symptom	Baseline Week (%)	Fourth Venlafaxine Week (%)	P*
Tiredness (fatigue)	48	8	.01
Dry mouth	30	19	.29
Sweating	69	38	.03
Trouble sleeping	52	22	.07
Nervousness	19	8	.25

*McNemar P value for association.

the development of prospective trials to evaluate three other selective serotonin re-uptake inhibitors (sertraline hydrochloride, paroxetine hydrochloride, and fluoxetine hydrochloride) for the treatment of hot flashes in breast cancer survivors. The anecdotal experiences that led to the development of these three trials, along with the data presented here, compellingly suggest that selective serotonin re-uptake inhibitors do represent a novel nonhormonal mechanism for treating hot flashes. Assuming that placebo-controlled evaluations of these compounds will confirm this, many questions are raised. Which of these drugs are most efficacious and least toxic? Which doses should be used? (The venlafaxine dose we used is only 10% to 20% of the dose usually used for the therapy of depression.) How long will the hot flashes be controlled? What is the mechanism of action?

While we fully understand the limitations of historical controls and of cross-study comparisons, the present trial was designed in a similar manner and used similar measurement techniques as those used in the previous trials we conducted.[2,5,7,8] The results from this comparison support the conduct of a placebo-controlled, double-blind clinical trial, similar to those we have previously conducted[2,5,7,8] to define better the efficacy and toxicity of venlafaxine in this setting. Such a trial is being developed and should soon be underway. This trial is designed to compare venlafaxine daily doses of 37.5 mg versus 75 mg versus 150 mg (all in a single daily dose sustained release preparation) with a placebo.

Regarding the mechanism of action of selective serotonin re-uptake inhibitors, we hypothesize that hot flash reduction by selective serotonin re-uptake inhibitors is associated with changes in central dopaminergic activity. Selective serotonin re-uptake inhibitors may alter dopaminergic pathways by inhibiting serotonin re-uptake, as evidenced by induction of hyperprolactinemia, galactorrhea, and extrapyramidal reactions in some individuals who receive these drugs.[10-12]

In addition to this evidence that selective serotonin re-uptake inhibitors alter dopaminergic activity, there also is evidence that both dopaminergic agents (eg, bromocriptine) and antidopaminergic agents (eg, metoclopramide and veralipride) can suppress hot flashes.[13-20] This confusing picture concerning hot flash reduction by both dopaminergic and antidopaminergic agents might be explained by differential abilities of individual drugs at crossing the blood brain barrier; and by whether individual drugs act on dopaminergic receptors in the hypophysis versus the pituitary (which is outside the blood-brain barrier) versus both.

There are still further data consistent with the hypothesis that alterations in central dopaminergic tone may effect the development of hot flashes. These data come from hypoestrogenic conditions in women, which are not associated with hot flashes. For example, hypoestrogenic women with pituitary prolactinomas (in whom central dopaminergic tone is presumed to be elevated[21] to compensate for elevated prolactin levels) do not experience the hot flashes that generally occur with low estrogen states. Further study is needed to support, or refute, our hypothesis that selective serotonin reuptake inhibitors suppress hot flashes through changes in central dopaminergic activity.

In summary, it now appears that selective serotonin re-uptake inhibitors will probably be helpful nonhormonal agents for treating bothersome hot flashes in cancer survivors. Additional studies that are either ongoing or in development will better address the pros and cons of using the newer classes of antidepressants as a means to alleviate hot flashes in cancer survivors.

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